REMARKS

Status of the Claims

Applicants first note that it appears to be in error that the Examiner mentions claims 10, 19-22 and 28 have been amended via the amendment filed 03/01/04. In fact, the above claims have been lastly amended via the amendment filed 11/07/05, which has been acknowledged by the Examiner in this Office Action.

Claim 10 has been amended to recite a method of treating a colon disorder of an individual by oral administration of a therapeutically effective amount of recombinant food grade gram-positive bacteria expressing trefoil peptides in the colon. Support for the amendments of "colon disorder" and "expressing trefoil peptides in the colon" is found in Examples 2 and 3, Figures 6A-6C, 7, 8A-8B and 11 of the instant specification. Support for the amendment of "an individual" is found in paragraphs [0051] and [0052] of the instant specification. Support for the amendment of "a therapeutically effective amount" is found in paragraph [0052] of the instant specification. Support for the amendment of "food grade gram-positive bacteria" is found in former claim 20, which has subsequently been cancelled.

New claims 30-32 have been added. Support for these new claims is found in paragraphs [0012], [0051] and [0075] of the instant specification.

Claims 11, 21-24, 27 and 28 have been amended for formality reasons that are not related to patentability.

Claims 19 and 29 have been cancelled.

Pursuant to 37 C.F.R. §1.118(a), Applicants respectfully submit that the above amendments do not introduce any new material into the application. With the present

amendments, 12 claims are pending in the application, namely, claims 10, 11, 21-24, 26-28 and 30-32.

Objection(s) to Specification

The Examiner objects to the specification for allegedly improper use of the trademarks as well as for allegedly failing to provide proper antecedent basis for the claimed subject matter. In response, Applicants have amended paragraphs [0061] and [0076] containing the use of the trademarks by referring properly to the registered trademarks as requested by the Examiner. In addition, Applicants have amended claim 10 by removing the previously added limitation of "subject" and replacing it with --individual--, for which the instant specification provides proper antecedent basis. Consequently, this objection to the specification is now overcome.

Rejection under 35 U.S.C. § 112, Second Paragraph

Claims 10, 11, 19-24 and 26-29 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. In response, Applicants have amended claims 10, 21-24, 27 and 28 to address the issues raised by the Examiner. It is believed that the present rejection is now overcome.

Rejection under 35 U.S.C. § 112, First Paragraph (Written Description)

Claim 10 and those dependent therefrom stand rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. In response, Applicants have amended claim 10 by removing the limitation of "subject" and replacing it with

--individual--. Direct written support for the presently added limitation is found in paragraphs [0051] and [0052] of the instant specification. Consequently, this rejection is now overcome.

Rejection under 35 U.S.C. § 112, First Paragraph (Scope of Enablement)

Claims 10, 11 and 19-29 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. Applicants respectfully traverse this rejection and present the following arguments.

Claim 10 has been amended to refer to a method of treatment of a colon disorder of an individual by orally administering to the individual a therapeutically effective amount of recombinant food grade gram-positive bacteria expressing trefoil peptides in the colon. With the presently added limitations of "colon disorder" and "a therapeutically effective amount of recombinant food grade gram-positive bacteria expressing trefoil peptides in the colon" (emphases added), Applicants believe that claims as presently amended are well enabled by the instant specification for the following reasons:

1. Colon Disorder

The Examiner states that the broadly recited "intestinal disorders" encompasses a plethora of intestinal disorders which are not limited to those with lesions or those localized to colon, or to acute colitis, ulcerative colitis, or Crohn's disease. In response, Applicants have amended claim 10 to recite "colon disorder" as per the Examiner's previously proposed draft amendment prior to issuing the present Office Action.

The Examiner also states that there is no indication in the instant specification that the DSS-induced acute colitis murine model is the art-accepted animal model for Crohn's disease or

colitis ulcerosa. Applicants submit that although there does not exist something like "the art-accepted animal model for Crohn's disease or colitis ulcerosa", many literatures suggest that DSS-induced colitis is a widely accepted model for making prognosis on human inflammatory bowel disease (IBD). As understood by one of ordinary skill in the art, the term "human inflammatory bowel disease" encompasses Crohn's disease (CD) and ulcerative colitis (UC). *See* a very recent review on mouse models of inflammatory bowel disease by Byrne and Viney (2006), a copy of which is enclosed herewith for Examiner's review and reference.

The state of the art suggests that DSS-induced acute colitis has become a relevant and accepted model for acute re-activation of Crohn's disease and ulcerative colitis, where epithelial damage is prominent. Applicants' instant study on treatment of DSS-induced acute colitis by oral administration of TFF-expressing recombinant lactobacteria was published in Gastroenterology, 127:502-513 (Vandenbroucke et al., 2004, a copy of which was previously provided to the Examiner) and highly recognized in the editorial section "This month in Gastroenterology" (on page 667 of the same issue):

> "These data therefore provide promise for the development of clinically useful and novel compounds that can further improve on management of acute and chronic colitis and epithelial damage in humans."

Other literatures that support Applicants' notion include the following: (a copy of each literature is enclosed herewith for Examiner's review and reference.)

McCole et al. (Gastroenterology, 2005) states: "(o)ne of the most commonly used models is the dextran sulfate sodium (DSS) model, which shares some similarity with human ulcerative colitis." *See* page 591, right column, second paragraph.

Hollenbach et al. (FASEB J., 2004) states: "(d)extran sulfate sodium (DSS)-induced colitis in mice shows reproducible morphological changes, which are very similar to those seen in patients with ulcerative colitis." *See* the bottom of page 1.

Forbes et al. (J. Immunology, 2004) states: "(s)everal experimental models of UC have been developed in mice to dissect out the key cellular and molecular mechanisms predisposing to disease ... In this study we have dissected out the potential contribution of eosinophils to the pathogenesis of UC by using a model of disease that is induced by dextran sulfate sodium (DSS). We show in this model that the administration of DSS induces a prominent colonic eosinophilic inflammation and GI dysfunction (diarrhea with blood and shortening of the colon) which resembles UC in patients." *See* page 5664, right column, third and fourth paragraphs.

Fujii et al. (Gut, 2004) states: "(t)here are many experimental animal models of human UC. In recent reports, colitis was induced mainly in mice and rats by colitis inducing agents such as dextran sulphate sodium (DSS) and trinitrobenzene sulphonic acid (TNB)." *See* page 710, right column, first paragraph.

Jeffers et al. (Gastroenterology, 2002) states: "we examined the in vivo effects of FGF-20 in 2 rodent models of IBD: dextran sulfate sodium (DSS) treatment of mice to induce an ulcerative colitis-like syndrome and indomethacin treatment of rats to induce ulceration and inflammation of the small bowel, as is seen in Crohn's disease." *See* page 1152, left column, second paragraph.

Jurjus et al. (J. Pharmacological and Toxicological Methods, 2004) provides a review on mouse models of inflammatory bowel disease (IBD), among which DSS colitis is more extensively discussed than any other models. *See* pages 87-89.

In view of the above literatures, Applicants believe that DSS-induced colitis murine model is representative of the full scope of Crohn's disease, ulcerative colitis, acute colitis and other colon disorders where epithelial damage is prominent.

2. Food Grade Gram-Positive Bacteria

The Examiner asserts that the broadly recited term "a recombinant microorganism" in claim 10 encompasses any microorganism other than *Lactococcus lactis*. In response, Applicants have amended claim 10 to recite "food grade gram-positive bacteria".

The Examiner further asserts that one recombinant trefoil peptide-expressing species of the genus *Lactococcus*, i.e., *Lactococcus lactis*, is not representative of the full scope of the large genus: 'a bacterium of a food grade gram-positive bacterial strain', '*Lactobacillus* species' and '*Lactococcus* species' by citing several references to support her position. Applicants respectively disagree.

Applicants submit that *Lactococcus* and *Lactobacillus* species, both of which are gram-positive, are related in both structure and physiology, which allows for a similar approach for trefoil peptide production *in vivo* when used as a carrier. One of ordinary skill in the art could readily place a TFF gene under the control of a constitutive promoter (e.g., the one provided in the present invention) and a secretion leader sequence (e.g., the one provided in the present invention) for production of trefoil peptides. Upon following the guidance provided in the

present invention, one of ordinary skill in the art could readily duplicate the same therapeutical effects as those achieved in the present invention when using other gram-positive bacterial hosts.

Applicants assert that the references cited by the Examiner do not apply to the present invention, wherein orally administered recombinant food grade gram-positive bacteria express trefoil peptides in the diseased colon tissues (emphasis added) and the expressed trefoil peptides directly (emphasis added) serve as therapeutical agents to treat the diseased colon tissues. That is, the treatment method claimed in the present invention does not involve antigen-antibody immune response for therapeutical effects. Rather, the *in vivo*-expressed trefoil peptides are themselves therapeutical agents and do not activate the immune system of the individual under the treatment to produce antibodies. As described in paragraph [0076] of the instant specification, the treated mice did not show an immune response towards the expressed proteins.

In contrast, the references cited by the Examiner either discuss *Escherichia coli* (i.e., gram-negative) as the host for production of foreign proteins, or describe lactobacteria as the vaccine vectors to express heterologous antigens *in vivo* so as to elicit or enhance an immune response. As known in the art, gram-negative bacteria produce endotoxins at high rates, which act differently from gram-positive bacteria. As to lactobacterial hosts for immunization, these hosts produce recombinant antigens which accumulate either inside the hosts or at the surface of the hosts. Immunization of animals with different bacterial hosts leads to different levels of systemic immune responses to the recombinant antigens, which is assumed to be the cause of different read-outs in immunization potential (i.e., variations in immune response) among different bacterial expression hosts.

As disclosed by Shaw et al. (WO 01/21200, cited by the Examiner), immunization through oral route is complex and involves appropriate responses of the entire immune system.

For oral immunization to be effective, sufficient antigens must be expressed *in vivo* and processed appropriately by the immunized animals before sufficient systemic immune response can be elicited. In particular, immunization process involves circulating IgG, which involves a complex cascade of events. Once inside the intestine, the antigens need to be presented to both B and T cells in an appropriate manner. The immune system then decides whether to elicit an immune response or to tolerate the antigens. If immunization is decided, T cells helps to properly activate primed B-cells. The activated primed B cells will then migrate out of the intestine to the liver and bloodstream where they can become IgG secreting plasma cells.

Other factors not described by Shaw will also lead to variations and unpredictability among various bacterial expression hosts, such as different surface structures on the host bacteria will activate the immune system differently; pre-exposure status of the animal to particular bacterial hosts prior to immunization may seriously change the outcome of immune response.

Applicants submit that it is not logical to compare the present invention with those references that teach immunization of animals with recombinant bacteria; as the present invention does not involve immunization. The present invention teaches localized delivery and immediate, direct action on the diseased tissues. In the instant method, the treatment of colitis only requires the *in situ* secretion of trefoil peptides by the genetically engineered bacteria. Through the oral route, the recombinant bacteria are brought in direct contact with the damaged epithelium of the diseased tissues. Upon release from the bacterial host, the trefoil peptides will immediately reach the final (emphases added) target cells, wherein effectors will be switched on and the curative effect achieved (Vandenbroucke et al., Gastroenterology 2004, a copy of which was previously provided to the Examiner.)

In view of the above remarks, Applicants believe that the instant therapeutical effects achieved from using *Lactococcus lactis* as the host for delivering and secreting trefoil peptides at the diseased tissues could readily be extrapolated to other food grade gram-positive bacterial trefoil peptide producers due to structural and physiological similarities shared among gram-positive bacteria. The Examiner has not provided an applicable reference to suggest otherwise.

3. Therapeutically Effective Amount

The Examiner states that there is no showing that one of skill in the art can practice the instantly claimed method of treatment by oral administration of 'a' (i.e., single) recombinant food grade gram-positive bacterial strain, and that a single Lactococcus lactis cell expressing 'a' trefoil peptide molecule is able to treat any of the 'intestinal disorders'. Furthermore, the Examiner states that none of the instant claims recite the quantity of orally administered recombinant bacteria expressing the trefoil peptides, which is an essential or critical feature of the present invention.

In response, Applicants have amended claim 10 by incorporating the limitation of "a therapeutically effective amount" of recombinant bacteria expressing trefoil peptides, and added dependent claim 30 to refer to a preferred dosage range which is considered to be therapeutically effective. The term "therapeutically effective amount" is clearly defined in paragraph [0052] of the instant specification as being such which is sufficient to show benefit to the patient. Such benefit may be at least amelioration of one symptom. The actual amount administered will depend on the aim of the administration, e.g. the biological effect sought in view of the nature and severity of the challenge, and is the subject of routine optimisation. The preferred dosage

range of at least 10^8 recombinant bacteria is demonstrated as the best mode to practice the present invention.

In view of the above remarks, Applicants believe that the claims as presently amended are well enabled by the instant specification. Accordingly, the present non-enablement rejection should be traversed.

*****

This document is filed timely and no fee is believed to be due. However, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, the Commissioner is authorized to deduct said fees from Howrey Deposit Account 08-3038/13475.0002.PCUS00.

Respectfully submitted,

J. Wendy Davis, Ph.D.
Reg. No. 46,393
Agent for Assignee
VLAAMS INTERUNIVERSITAIR INSTITUUT
VOOR BIOTECHNOLOGIE

Customer No. 23369
HOWREY LLP
1111 Louisiana, 25th Floor
Houston, Texas 77002
(713) 787-1400

Date: May 5, 2006